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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 18377-0031	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/US04/10121	International filing date *(day/month/year)* 31 March 2004 (31.03.2004)	Priority date *(day/month/year)* 31 March 2004 (31.03.2004)
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 5/00, 15/85, 15/86; A01N 1/02 and US Cl.: 435/325, 1.1, 405		
Applicant BRESAGEN INC.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26 April 2005 (26.04.2005)	30 September 2005 (30.09.2005)
Name and mailing address of the IPEA/ US Mail Stop PCT, Attn: IPEA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703) 305-3230	Authorized officer Joseph T. Woitach Janice Ford for Telephone No. (571) 272-1600

Form PCT/IPEA/409 (cover sheet)(April 2005)

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International application No.
PCT/US04/10121

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:
   - ☐ the international application in the language in which it was filed.
   - ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:
     - ☐ international search (under Rules 12.3 and 23.1(b))
     - ☐ publication of the international application (under Rule 12.4(a))
     - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:
   - ☐ the international application as originally filed/furnished
   - ☒ the description:
     pages 1-78 as originally filed/furnished
     pages* NONE received by this Authority on _____
     pages* NONE received by this Authority on _____
   - ☒ the claims:
     pages NONE as originally filed/furnished
     pages* NONE as amended (together with any statement) under Article 19
     pages* 79-84 received by this Authority on 26 April 2005
     pages* NONE received by this Authority on _____
   - ☒ the drawings:
     pages 1-23 as originally filed/furnished
     pages* NONE received by this Authority on _____
     pages* NONE received by this Authority on _____
   - ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages _____
   - ☐ the claims, Nos. _____
   - ☐ the drawings, sheets/figs _____
   - ☐ the sequence listing (*specify*): _____
   - ☐ any table(s) related to the sequence listing (*specify*): _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages _____
   - ☐ the claims, Nos. _____
   - ☐ the drawings, sheets/figs _____
   - ☐ the sequence listing (*specify*): _____
   - ☐ any table(s) related to the sequence listing (*specify*): _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

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Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 42-43, 86-87	YES
	Claims 1-41, 44-85	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-87	NO
Industrial Applicability (IA)	Claims 1-87	YES
	Claims NONE	NO

2. Citations and Explanations (Rule 70.7)

Claims 1-41 and 44-85 lack novelty under PCT Article 33(2) as being anticipated by US 2002/0164794 (Wernet), US 2002/0160510 (Hariri), US 6,200,806 (Thomson), US 6,280,718 (Kaufman et al.), or US 5,842,780 (Thomson).

The claims encompass stem cells and specific uses of stem cells for treatment of neuronal disorders. At the time of filing each US 2002/0164794 (Wernet), US 2002/0160510 (Hariri), US 6,200,806 (Thomson), US 6,280,718 (Kaufman et al.), or US 5,842,780 (Thomson) disclose pluripotent stem cells and the methods required to obtain said cells. While it is desirable to maintain the correct karyotype of a stem cell for further research and for use in potential treatments, each disclose that culturing can randomly alter the genome of the isolated cells. It is taught that many different karyotypes can be identified in addition to the desired normal karyotype, and that isolation and identification of single cell clones must be done on each isolate for determination of the exact make-up of the genome.

Claims 1-87 lack an inventive step under PCT Article 33(3) as being obvious over US 2002/0164794 (Wernet), US 2002/0160510 (Hariri), US 6,200,806 (Thomson), US 6,280,718 (Kaufman et al.), or US 5,842,780 (Thomson).

As discussed above, each of the cited references provides the guidance to obtain and culture pluripotent stem cells. Each propose that the stem cells have great potential for use in therapy methods where restoration of a specific differentiated cell type through the differentiation capacity of the stem cell can be used in methods of treatment. It would have been obvious to use the cells of each US 2002/0164794 (Wernet), US 2002/0160510 (Hariri), US 6,200,806 (Thomson), US 6,280,718 (Kaufman et al.), or US 5,842,780 (Thomson) for use in methods of treating neurological disorders in which undifferentiated fetal cells have already been used.

Claims 1-87 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.

The claims encompass methods and uses of stem cells which is an active area of research.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-87 are objected to as lacking clarity under PCT Rule 66.2(a)(v) because the claims are not fully supported by the description. The application, as originally filed, did not describe pluripotent stem cells with the specific markers claimed, or provide the required guidance for the successful use. The art of record clearly indicates that the cell surface markers indicated to be lacking form the stem cell are a hallmark of a highly pluripotent stem cell. Importantly, the claims and disclosure specifically contemplate isolation from cell types previously taught in the art to comprise the cell surface markers. For example, comparing the disclosures of Thomson ('780 or '806) the first successfully disclosure of human stem cells demonstrates the pluripotent stem cells have have SSEA-4 and TRA-1.

In addition, it is noted that the karyotype of any cell in culture is a consequence of random alterations of the genome, and while the characterization of one line with a given genotype provides support for the fact that such a cell could be made, the disclosure fails to provide methodology to actively and specifically obtain a given genotype. While one may screen resulting cells for the presences of a genotype, the random nature of the process makes one disclosure insufficient as evidence that the cell will ever be produced again. Finally, in cells with dramatically affected karyotypes often the cell takes on

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Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

a. type of material

☒ a sequence listing

☒ table(s) related to the sequence listing

b. format of material

☒ on paper

☒ in electronic form

c. time of filing/furnishing

☒ contained in the international application as filed

☒ filed together with the international application in electronic form

☐ furnished subsequently to this Authority for the purposes of search and/or examination

☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

CLAIMS

WE CLAIM:

1. A human pluripotent embryonic stem cell culture, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
2. The cell culture of Claim 1, wherein the cell culture was dissociated to an essentially single cell culture.
3. The cell culture of Claim 2, wherein a majority of the cells have an abnormal karyotype.
4. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
5. The cell culture of Claim 4, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
6. The cell culture of Claim 5, wherein the autosomal chromosome is chromosome 12 or 17.
7. The cell culture of Claim 3, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
8. The cell culture of Claim 7, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
9. The cell culture of Claim 8, wherein the autosomal chromosome is chromosome 12 or 17.
10. A method of culturing a human pluripotent embryonic stem cell comprising,
    a) selecting a human pluripotent cell using an anti-SSEA4 antibody; and
    b) maintaining a culture of the cell by passaging the cell using a protease treatment, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.
11. The method of Claim 10, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.

12. The method of Claim 10, wherein the cell is maintained by using a protease treatment for at least 13 passages.
13. The method of Claim 10, wherein a majority of the cells of the culture have an abnormal karyotype.
14. The cell culture of Claim 13, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.
15. The cell culture of Claim 14, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
16. The cell culture of Claim 13, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.
17. The cell culture of Claim 16, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.
18. The method of Claim 11, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.
19. A method of providing a human cell culture enriched in neural cells, comprising forming an embryoid body comprising the human pluripotent embryonic stem cell of Claim 10.
20. The method of Claim 19, wherein the embryoid body is formed by culturing the cell with an essentially serum free medium.
21. The method of Claim 20, wherein the essentially serum free medium is a MEDII conditioned medium.
22. The method of Claim 21, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.
23. The method of Claim 21, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.
24. The method of Claim 23, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50 μM.
25. The method of Claim 19, wherein the embryoid body is formed by culturing the cell with a minimal medium.
26. The method of Claim 25, wherein the minimal medium is essentially proline free.

27. The method of Claim 25, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.

28. The method of Claim 0, wherein the minimal medium comprises proline at a concentration from approximately 50 μM to approximately 250 μM.

29. The method of Claim 25, wherein the minimal medium is essentially FGF free.

30. The method of Claim 25, wherein the minimal medium is essentially MEDII free.

31. A human pluripotent cell produced by the method of Claim 10.

32. A human cell culture enriched in neural cells, produced by any the method of any one of Claims 19-30.

33. The human cell culture of Claim 32, wherein greater than approximately 80% of the human cell culture comprises neural cells.

34. The human cell culture of Claim 33, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.

35. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 32.

36. The method of Claim 35, wherein the neural disease is Parkinson's disease.

37. A method of culturing a human pluripotent embryonic stem cell comprising,

a) providing a human pluripotent embryonic stem cell culture;

b) passaging the cell culture using a protease treatment to thereby disperse the cell to an essentially single cell culture; and

c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium

to thereby culture the human pluripotent embryonic stem cell.

38. The method of Claim 37, wherein the protease treatment comprises the sequential use of Collagenase and trypsin.

39. The method of Claim 38, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.

40. The method of Claim 37, wherein the feeder cell is a freshly plated feeder cell.

41. The method of Claim 40, wherein the feeder cell is a mouse embryonic fibroblast.

42. The method of Claim 40, wherein the feeder cell has been plated for less than 10 hours.

43. The method of Claim 40, wherein the feeder cell has been plated for less than 6 hours.

44. The method of Claim 40, wherein the feeder cell has been plated for less than 2 hours.

45. A human pluripotent embryonic stem cell culture produced by the method of Claim 37, wherein the cells of the culture do not express SSEA1, express SSEA3, SSEA4, Oct4, Tra-1-60, Tra-1-80, and express nestin substantially uniformly.

46. The human pluripotent cell embryonic stem culture of Claim 45, wherein a majority of the cells of the culture have an abnormal karyotype.

47. The human pluripotent embryonic stem cell culture of Claim 46, wherein the abnormal karyotype comprises a trisomy of at least one autosomal chromosome.

48. The human pluripotent embryonic stem cell culture of Claim 47, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.

49. The human pluripotent embryonic stem cell culture of Claim 46, wherein the abnormal karyotype comprises a trisomy of more than one autosomal chromosome.

50. The human pluripotent embryonic stem cell culture of Claim 49, wherein the autosomal chromosome is selected from the group consisting of chromosomes 1, 7, 8, 12, 14, and 17.

51. A method of producing a human pluripotent embryonic stem cell culture enriched in neural cells comprising,

a) providing a human pluripotent embryonic stem cell culture;

b) passaging the cell culture using a protease treatment to thereby disperse the cell culture to an essentially single cell culture;

c) culturing the essentially single cell culture in the presence of a feeder cell, a conditioned medium, or a minimal medium; and

d) forming an embryoid body comprising the essentially single cell culture by culturing the cell culture with an essentially serum free medium, .

to thereby produce the human cell culture enriched in neural cells.

52. The method of Claim 51, wherein protease treatment comprises the sequential use of Collagenase and trypsin.

53. The method of Claim 52, wherein Collagenase is used at a concentration of approximately 1 mg/ml for approximately 5 minutes, and wherein trypsin is used at a concentration of approximately 0.05% for approximately 30 seconds.

54. The method of Claim 51, wherein the essentially serum free medium is a MEDII conditioned medium.

55. The method of Claim 54, wherein the MEDII conditioned medium is a Hep G2 conditioned medium.

56. The method of Claim 54, wherein the MEDII conditioned medium comprises one or more proline residues or a polypeptide containing proline residues.

57. The method of Claim 56, wherein the MEDII conditioned medium comprises proline at a concentration of approximately 50 μM.

58. The method of Claim 51, wherein the feeder cell is a freshly plated feeder cell.

59. The method of Claim 58, wherein the feeder cell is a mouse embryonic fibroblast.

60. The method of Claim 58, wherein the feeder cell has been plated for less than 10 hours.

61. The method of Claim 58, wherein the feeder cell has been plated for less than 6 hours.

62. The method of Claim 58, wherein the feeder cell has been plated for less than 2 hours.

63. The method of Claim 51, wherein the minimal medium comprises one or more proline residues, or a polypeptide containing proline residues.

64. The method of Claim 63, wherein the minimal medium comprises proline at a concentration from approximately 50 μM to approximately 250 μM.

65. The method of Claim 51, wherein the minimal medium is essentially proline free.

66. The method of Claim 51, wherein the minimal medium is essentially FGF free.

67. The method of Claim 51, wherein the minimal medium is essentially MEDII free.

68. A human cell culture enriched in neural cells produced by the method of Claim 51.

69. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the neural cell of Claim 68.

70. The method of Claim 69, wherein the neural disease is Parkinson's disease.

71. The human cell culture of Claim 68, wherein greater than approximately 80% of the human cell culture comprises neural cells.

72. The human cell culture of Claim 71, wherein greater than approximately 90% of the neural cells express tyrosine hydroxylase.

73. A method for treating a patient, comprising a step of administering to the patient having a neural disease a therapeutically effective amount of the human cell culture enriched in neural cells of Claim 68.

74. The method of Claim 73, wherein the neural disease is Parkinson's disease.